Corrected

"A Study on Antioxidant Potential of Soy Isoflavone Daidzein in Human Ovarian Cancer Cell Lines".

SYNOPSIS

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Lalit Narayan Mithila University, Darbhanga

Submitted by

Archana 20/8/25

Archana

Research scholar

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Under the Guidance of

20/8/25

Dr. Shushovan Mallik

Assistant professor

University Department of Zoology

L.N. Mithila University ,Darbhanga

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1. "A study on antioxidant potential of soy isoflavone Daidzein in human ovarian cancer cell lines".

The recent advances in understanding of free radicals and Reactive Oxygen Species (ROS) are emerging as a scientific revolution that promises a new transformation of health and illness control. Electromagnetic radiation, cosmic radiation, UV-light, ozone, cigarette smoking and low wavelength electro-magnetic radiation transport chain and β-oxidation of fat are examples of external sources of reactive oxygen species. Oxidants on the other hand, assault macromolecules such as protein, DNA, and lipids, causing cellular/tissue damage. Antioxidants are chemicals that dispose free radical species, scavenge them, suppress their synthesis of formation of those free radicals, or oppose their action. There is an optimal Pro-oxidant vs. Antioxidant equilibrium in a normal cell. When the production of oxygen species is boosted or antioxidant levels are depleted, how-ever this equilibrium can be changed towards the pro-oxidant. This is referred to as "oxidative stress," and it can cause substantial cell damage if the stress is severe or continuous. Exogenous and endogenous antioxidants typically work together to achieve a balance between protection against reactive oxygen species which is thought to be vital in maintaining a healthy biological system (Dias TR, *et al.*, 2020). Increased levels of oxidants/Reactive Oxygen Species (ROS) make a path for

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oxidative stress, which has been linked to the creation of a wide range of chronic illnesses, including cardiovascular and periodontal disorders (Pampani P, *et al.*, 2021). Herbal antioxidants have been successfully used as rejuvenators in Indian systems of alternative medicine for several millennia. Several studies have demonstrated that various herbal remedies include a variety of compounds, many of which have antibacterial and radical scavenging characteristics that can protect the human body against infections as well as cellular oxidation events (Parham S, *et al.*, 2020).

There has been an extensive research regarding the curative and preventive potencies of various targeted molecules of dietary plant origin like Genistein, Diazdin, Lycopene, Emodin, Allicin, Vanillium etc. These have shown great actions to cure dreaded diseases that either caused death or lead to several dreaded side effects when treated with synthetic drugs. The herbal or phyto- chemicals now known as Nutraceuticals contain selective combinations of specific plant-derived bioactive components with renowned medicinal, disease-preventing, and/or health-enhancing properties. Such compounds include polyphenols, carotenoids, flavonoids, isoflavonoids, terpenoids, glucosinolates, phytoestrogens, and phytosterols. Studies on these phytochemicals have also shown positive pharmacological activities in human health (D. Prakash and C. Gupta, 2017).

Among the well studied phytochemicals obtained from soybeans and other leguminous plants are the isoflavonoid compounds called Genistein and Daidzein (K. Zaheer and A. M. Humayoun

2017). Owing to the optimal hydroxylation patterns in their chemical structure which provides them with the ability to bind to Estrogens receptors, they are referred to as phytoestrogens (Setchell KD, 1998).

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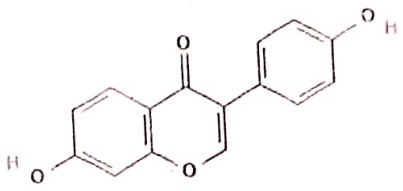
Daidzein [7-hydroxy-3-(4- hydroxyphenyl)-4H-1-benzopyran-4-one] is a naturally occurring soy derived phytochemical fits into the category of nonsteroidal phytoestrogen (A. Cassidy et al, 2003), with numerous pharmacological potentials such as anti-hemolytic activities, antioxidant activities and anti-inflammatory activities (S. A. Bingham, et al., 1998), (K. Dwiecki, et al., 2009).

Chemical structure



Preferred IUPAC name

(7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one)

Systematic name

4,7-Dihydroxyisoflavone

The chemical structure of Daidzein is analogous to mammalian estrogens, making it a promising candidate for a dual purpose by substituting/hindering such hormones and their corresponding receptors. Hence, Daidzein could be a future therapeutic strategy for estrogen-dependent health conditions such as breast (N. Sathyamoorthy and T. T. Wang, 1997) and prostate (M. Adjakly et al., 2013) cancer, diabetes, osteoporosis, and cardiovascular disease (CVD) (D. C. Vitale et al., 2013). However, Daidzein also has other estrogen receptor- (ER-) independent biological activities, like the ability to reduce oxidative damage, regulate the

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immune reaction (M. Masilamani et al., 2012) and induce apoptosis show a correlation with its anticancer potential (F. H. Lo et al 2007). Such activities, along with minimum toxicity features, make Daidzein a plausible compound for anticancer drug design. In this sense, the present study aims at providing an overview of Daidzein's potential to prevent or treat some burdening human health conditions by way of analyzing the antioxidant activity of Daidzein in cancer cell lines.

Incidence and mortality rates of hormone dependent tumors such as breast, prostate and ovarian cancer are considerably lower in Asia when compared to Western countries which been attributed to the higher soy consumption in the Asian population and has increased the interest of the scientific community throughout the globe to study the ability of soy isoflavones in prevention and treatment of such types of cancers (C. Nagata et al., 2014). Most studies show a dual effect of isoflavones on cancer depending on their concentration. Tissue distribution and concentration dependent studies provide an insight towards understanding whether Daidzein may have beneficial or harmful effects in cancer (S. M. Mense2008). Bolca et al. 2010, analyzed the concentration of isoflavones in normal breast tissue following a dietary intervention increasing isoflavone intake, and found that isoflavones may reach significant levels in breast to elicit a beneficial effect. Several in vivo studies have described an anticancer effect for Daidzein in different types of tumors (J. M. Guoet al., 2004) and have revealed that Daidzein could induce apoptosis and cell cycle arrest in the SKOV3 ovarian cancer cell line (F. Hua, 2018) or induce epigenetic changes (G. L. Russo and P. Ungaro, 2019). Furthermore, Daidzein treatment was found to modulate long non-coding RNA (lnc RNA) expression in some cancer types, as several isoflavones have been reported to target these molecules (S. Mishra et al.,2019).

2. PURPOSE OF RESEARCH:

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- To determine enzymatic and non enzymatic antioxidant activity in cultured human cancer cell lines on Daidzein treatment.
- To analyze the mechanism of induction of apoptosis in cancer cells on treatment with Daidzein.
- To evaluate the effects of Daidzein on oxidant induced damage of DNA in the cultured cell lines.
- To ascertain the potential of Daidzein against cancer or itself can act as a good therapeutic agent cancer.

3. A BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD:

A number of pharmacological activities have been accounted for daidzein, which includes anti-carcinogenesis, anti-fibrotic, anti-diabetic, cholesterol-lowering and cardiovascular activity. Daidzein pretreatment was found to diminish the seriousness of mucosal damage in a portion subordinate way. Some other therapeutic uses of daidzein are weight reduction, decreasing bowels moment, and inflammation associated with histopathological deformities (Amaral et al., 2017). Their structure and function are alike to human estrogen, which can play a massive role in the prevention of osteoporosis, cancer, and postmenopausal syndromes. Uses of daidzein in anticancer activity against ovarian cancer are still limited (Meng et al., 2017). Epidemiological data suggest that increased utilization of soybean in food results in decreased cancer risk. Soybean food is highly recommended in cancer prevention because it has a number of anticarcinogens (Yu et al., 2017). The potent antioxidant activity of Daidzein reported in various *in vitro* and *in vivo* studies was conclusively by many workers worldwide.

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It was shown that Daidzein could inhibit 5-FU-induced intestinal mucositis through prevention of relevant clinical symptoms, stimulation of the proliferative activity in the epithelium and crypts, suppression of oxidative stress and regulation of inflammatory homeostasis (Atiq et al., 2019). Furthermore studies showed that Soluble CXCL16 and VEGF-A are two promoters of angiogenesis and metastasis in breast cancer. The stimulation of these two angiogenesis-related cytokines might represent one of the mechanisms explaining the proliferative effects of low isoflavone doses in estrogen-dependent cells. In estrogen-independent cells, daidzein inhibited their secretion, indicating anti-angiogenic properties (Uifalean et al., 2018).

It was also shown that the utilization of Daidzein is associated with a reduced risk of prostate cancer but the direct effects of Daidzein on the androgen receptor (AR) signaling pathway are not well understood (Sivoňová et al., 2019). In connection to the above it was found that Cytotoxic effects of daidzein on both MIA PaCa-2 and HT- 29 cell lines occurred in a dose-dependent manner. Daidzein exhibits genotoxic potential under *in vitro* conditions (Gundogdu et al., 2018). Genistein and daidzein inhibit the accumulation of lipid droplets (LDs) by regulating LDs-related factors and lead to final apoptosis of colon cancer cells and possible molecular mechanisms of isoflavones in anti-obesity and anti- tumor functions (Liang et al., 2018).

Furthermore Genistein, daidzein, and ERB-041 decreased ovarian cancer cell migration, invasion, proliferation, and bubble formation, signifying their roles on ovarian cancer cell metastasis, tumorigenesis, and their potential as other therapies for ovarian cancer patients(Chan et al., 2018). Daidzein was found to enhance cisplatin-impaired antioxidant defense such as reduced glutathione reserve, GPX activity and SOD activity(Meng et al., 2017). Studies also showed that Anti-proliferative action of 12 anti-carcinogens from

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soybean against MCF-7 and MDA-MB-231 human breast cancer cell outline by single or two-way combination treatment. There is a synergistic result of the combination treatment of genistein plus daidzein MCF-7 cells (Zhu et al., 2018).

4. PROPOSED METHODOLOGY IN BRIEF:

The study would encompass treatment of Diazdein to cultured liver cancer cell lines and analysis of various parameters in comparison to untreated control.

A. Antioxidant Analysis

i. Enzymatic antioxidant assay

ii. Non enzymatic antioxidant assay

B. Cytotoxicity Assay

C. Biochemical Assays

D. Statistical Analysis of results would be done using a suitable test of significance.

5. EXPECTED OUTCOME OF THE PROPOSED WORK:.

The study would help in understanding the antioxidant potential of Daidzein which would be a prelude for understanding the mechanisms of interaction of Daidzein with cellular molecules like DNA and proteins which are the key factors responsible for regulation of cell cycle and progression of cancer. The study would facilitate in designing the alternative drugs and to decide accurate delivery systems to treat cancer. The present study intends establish Daidzein as a candidate drug in the prevention and prognosis of cancer.

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Signature of scholar

signature of supervisor

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